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# An Inherited Mutation Associated with Functional Deficiency of the α-Subunit of the Guanine Nucleotide-Binding Protein $G_s$ in Pseudo- and Pseudopseudohypoparathyroidism*

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**ABSTRACT**

Pseudohypoparathyroidism type Ia (PSP) is a disorder characterized by Albright's osteodystrophy, secondary hyperparathyroidism, lowered $G_s$ activity, and resistance of the urinary cAMP excretion to exogenous PTH. The patients had raised basal serum levels of TSH and/or excessive TSH response to TRH. Here we have described a 38-bp deletion at the exon 1/intron 1 boundary of one $G_s\alpha$ allele in two mothers with pseudo-PSP and in six offsprings with PSP of a kindred with Albright's osteodystrophy. The deletion eliminates the splice donor site of exon 1. The pseudo-PSP patients presented decreased $G_s$ activity, but normal urinary cAMP responses to PTH and normal TSH levels and responses to TRH. As monitored during 22 yr, they had normal serum levels of calcium and PTH. The findings demonstrate the same inherited functional defect of $G_s\alpha$ in two female patients with pseudo-PSP and in six of their offspring with PSP. The pathogenesis of clinical hypoparathyroidism remains to be clarified. (*J Clin Endocrinol Metab* **83:** 935–938, 1998)

PSEUDOHYPOPARATHYROIDISM Ia (PSP) is a disorder with clinical hypoparathyroidism and the phenotype of Albright's osteodystrophy, but raised plasma levels of bioactive and immunoreactive PTH (1; see Ref. 2 for additional references). Patients with PSP frequently present raised serum levels of TSH and excessive TSH response to TRH with sometimes overt clinical hypothyroidism (3 and references cited therein). The diagnosis of PSP-Ia was established on the basis of resistance of the urinary cAMP excretion to exogenous PTH, and decreased guanine nucleotide-binding protein $G_s$ activity (4–10). $G_s$ activity was decreased in the PSP patients reported here, but it was similarly decreased in their mothers with pseudo-PSP (8). The latter responded normally to exogenous PTH with raised urinary cAMP excretion; secondary hyperparathyroidism and excessive TSH response to TRH were not observed. A functional deficiency of $G_s$ has been reported in PSP and pseudo-PSP (8–10).

A genetic deficiency of $G_s\alpha$ has been revealed in familial PSP and pseudo-PSP (11–15; for additional mutations, see Ref. 16). As shown here, the patients with PSP-Ia as well as those with pseudo-PSP carry a novel deletion in one $G_s\alpha$ allele resulting in reduced $G_s\alpha$ activity. In healthy siblings with no signs of Albright's osteodystrophy, both $G_s\alpha$ alleles were normal. It would seem, therefore, that the described genetic defect, resulting in reduced $G_s\alpha$ activity, is not an obvious cause of the resistance to exogenous PTH in the kindred reported here.

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## Subjects and Methods

Figure 1 shows the pedigree of a family with PSP, pseudo-PSP, and Albright's osteodystrophy. Laboratory data, including serum calcium, serum PTH, urinary cAMP responses to parathyroid extract (Eli Lilly Co., Indianapolis, IN), serum TSH, TSH response to TRH, and $G_s$ protein activity, for patients 7, 9, 14–17, 20, and 22 have been reported previously (3, 5, 8) (Table 1).

Briefly, serum levels of calcium were measured by ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*′-tetraacetate titration, using calcein as an indicator, and by flame spectrophotometry (6, 9). Immunoreactive serum PTH was estimated with antibodies to bovine PTH recognizing predominantly intact human PTH-(1–84) in the serum of patients with PSP on gel permeation chromatography. [$^{131}$I]Bovine PTH-(1–84) was used as radioligand, and human PTH-(1–84) as standard (8, 17). The urinary cAMP response to parathyroid extract was evaluated according to a slightly modified protocol of Chase *et al.* (4, 5). Immunoreactive TSH and the TSH response to TRH (Hoffmann-La Roche, Basel, Switzerland) were estimated as previously reported (3). $G_s$ protein activity was assessed in erythrocyte ghosts as previously described (8).

Genomic DNA isolated from peripheral leukocytes of individual subjects was analyzed for $G_s\alpha$ gene insertions or deletions by PCR amplification of gene subdomains. The primer pairs indicated in Fig. 2 were used to localize and analyze in detail the deletion described here in exon 1 and flanking regions of the $G_s\alpha$ gene. PCR products were separated by electrophoresis in 2% agarose (Seakem HGT, Flowgen Instruments, Sittingbourne, UK) and visualized by

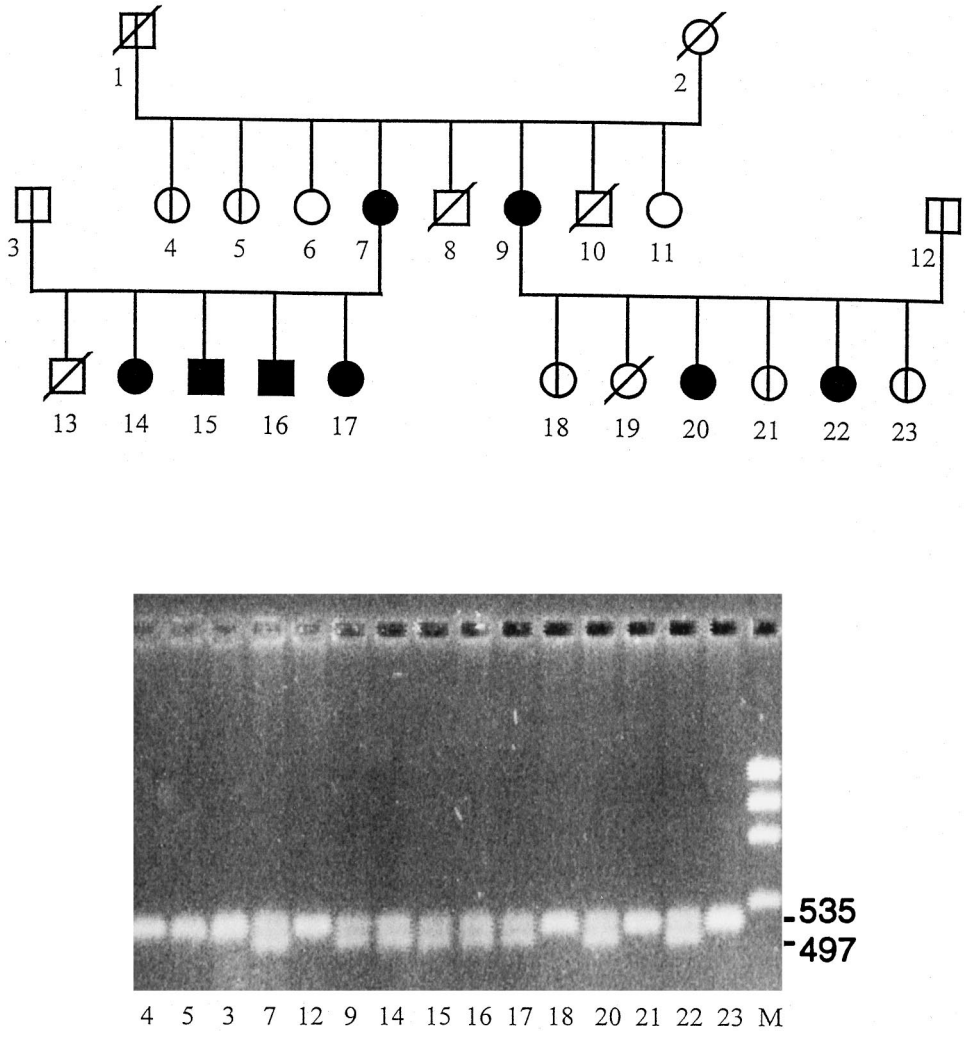


FIG. 1. Pedigree of a family with (■ and ●) and without (□ and ○) Albright's osteodystrophy. □ and ○, Not tested. *Diagonal lines* denote deceased subjects (*top*). Results of agarose gel electrophoresis analysis of DNA products obtained by PCR amplification of DNA isolated from blood samples of selected family members (*bottom*). The numbers below individual lanes of the agarose gel correspond to the numbers of individual family members, as indicated in the pedigree. M, DNA size markers (ΦX/*Hae*III).

ethidium bromide staining. Nucleotide sequence analysis of PCR-amplified $G_s\alpha$ gene fragments was performed by cycle sequencing. The primers used were the same as those used for amplification of gene fragments.

## Results

Figure 1 shows the pedigree of the affected family with Albright's osteodystrophy. At first presentation, patients 14–17, 20, and 22 suffered from PSP, with hypocalcemia in four and normocalcemia in two siblings (Table 1). Serum PTH levels were raised, and the patients were classified as PSP-I on the basis of absent or low urinary cAMP responses to exogenous PTH. Five of the six siblings presented raised levels of TSH, and all had excessive TSH response to TRH. $G_s$ activity was decreased in all of them, but $G_s$ was also decreased in patients 7 and 9 with pseudo-PSP exhibiting normal urinary cAMP excretion in response to the administration of parathyroid extract and normal serum levels of TSH and TSH response to TRH at first presentation. They had normal serum levels of calcium and PTH as observed over 22 yr.

Analysis by PCR amplification of exon 1 and flanking regions of the $G_s\alpha$ gene with primers DV-157 and MET1R (Fig. 2) revealed a normal-sized 535-bp product in all family members investigated (Fig. 1). In the 6 patients with PSP and the 2 patients with pseudo-PSP, an additional smaller PCR product, indicating a deletion in 1 $G_s\alpha$ allele, was also observed. Subsequent separate PCR and nucleotide sequence analysis of the 5′- and 3′-ends of exon 1 and corresponding flanking regions with primer pairs DV-157/MAL-2 and MET1F/MET1R, respectively, revealed a 38-bp deletion comprising 21 nucleotides of the 3′-end of exon 1 and 17 nucleotides of intron 1 in the mutated allele. This eliminates the donor splice site of exon 1, giving rise to a transcript that includes intron 1. As a result, termination of translation is predicted to occur within intron 1 leading to the incorporation of at least 116 alternative amino acids into a protein product of the mutated $G_s\alpha$ gene.

## Discussion

Albright *et al.* (1) made the discovery that PSP is not caused by a lack of PTH, but by an inability to respond to it. This has led to the concept of end-organ resistance to exogenous PTH caused by a defect of the PTH receptor and/or its signaling pathways. Mutations of the PTH/PTHrP receptor have not been detected to date in PSP type Ib (18–20) and have not

**TABLE 1.** Summary of laboratory data

| Subjects | Age at first presentation (yr) | Serum | | cAMP (nmol/min · $m^2$) | | TSH ($\mu$U/mL) | | $G_{s\alpha}$ (%) |
|---|---|---|---|---|---|---|---|---|
| | | Calcium (mmol/L) | PTH (ngeq/L) | Basal | Maximal | Basal | Maximal | |
| 1 | Deceased (age unknown) | — | — | — | — | — | — | — |
| 2 | Deceased (age unknown) | — | — | — | — | — | — | — |
| 3 | 47 | 2.27 | <100 | — | — | — | — | — |
| 4 | 56 | 2.24 | 100 | — | — | — | — | — |
| 5 | 54 | 2.36 | <200 | — | — | — | — | — |
| 6 | 52 | — | — | — | — | — | — | — |
| 7 | 36 | 2.20 | 220[a] | 4 | 90 | 0.9 | 13.8 | 60 |
| 8 | Deceased (10 days) | — | — | — | — | — | — | — |
| 9 | 34 | 2.35 | 135[b] | 4 | 226 | 1.7 | 13.0 | 55 |
| 10 | Deceased (1.17 yr) | — | — | — | — | — | — | — |
| 11 | 42 | — | — | — | — | — | — | — |
| 12 | 49 | 2.45 | <200 | — | — | — | — | — |
| 13 | Deceased (age unknown) | — | — | — | — | — | — | — |
| 14 | 16 | 2.05 | 1840 | 1.1 | 11.6 | 13.1 | 25.0 | — |
| 15 | 6 | 1.58 | 341 | 2.7 | 6.0 | 2.0 | 28.9 | 50 |
| 16 | 5 | 1.35 | 390 | 0.2 | 5.1 | 12.2 | 18.8 | 47 |
| 17 | 13 | 2.00 | 480 | 3.1 | 9.4 | 9.5 | 33.2 | — |
| 18 | 21 | 2.30 | 281 | — | — | — | — | — |
| 19 | Deceased (1 yr) | — | — | — | — | — | — | — |
| 20 | 5 | 2.43 | 390 | 3.8 | 1.4 | 7.0 | 32.4 | 39 |
| 21 | 26 | 2.31 | 327 | — | — | — | — | — |
| 22 | 1 | 2.45[a] | 270[c] | 4.7 | 11.0 | 7.6 | 39.4 | 51 |
| 23 | 12 | 2.36 | <200 | — | — | — | — | — |
| Control subjects (range) | | 2.08–2.43 | <300 | 0.3–5.9 | 17–268 | <1.0–3.3 | 10.0–18.0 | 91–113 |

—, Not available.
[a] At 58 yr, serum calcium was 2.38 mmol/L and PTH was 267 ng/L.
[b] At 56 yr, serum calcium was 2.39 mmol/L and PTH was 190 ng/L.
[c] At 13 yr, serum calcium was 2.35 mmol/L, and PTH was 1640 ng/L.

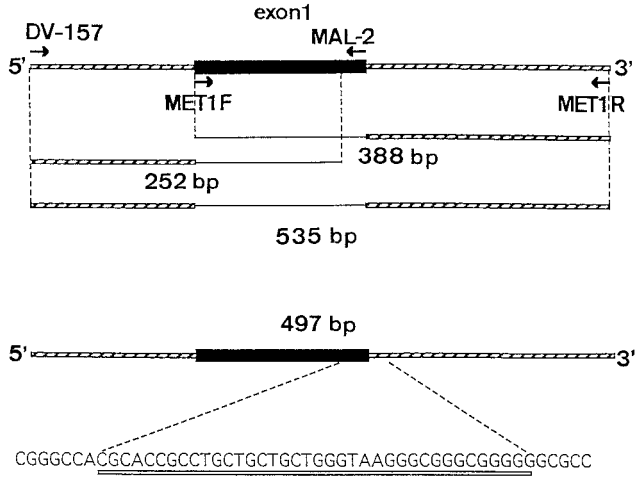


FIG. 2. Schematic illustration of the $G_s\alpha$ gene region analyzed by PCR with indicated forward (DV-157 and MET1F) and reverse (MAL-2 and MET1R) primers. The expected sizes (base pairs) of products amplified from a normal allele (*top*) and the location and sequence of the identified 38 nucleotide deletion (*underlined*; *bottom*) are shown.

been reported in PSP type Ia. The findings that urinary cAMP excretion in response to exogenous PTH is reduced or absent and of an excessive TSH response to TRH are consistent with inadequate activation of the PTH- and TRH-responsive adenylyl cyclase and different isotypes thereof, and activation of phosphodiesterase (4, 16). To this end, $G_s$ activity was shown to be reduced (7–11). Subsequently, several mutations of the $G_s\alpha$-encoding gene have been discovered (12–16). The mutations are equally present in patients with PSP and pseudo-PSP.

In the present report, a deletion in one $G_s\alpha$ allele has been identified in a large kindred with PSP, pseudo-PSP, and Albright's osteodystrophy, but not in unaffected siblings. Formation of an inactive protein with an altered carboxyl-terminus is predicted. Maintenance of approximately 50% $G_s$ activity in PSP and pseudo-PSP is provided by the normal allele. The findings resemble those obtained in mouse embryonic stem cells, in which one $G_s\alpha$ allele was disrupted by homologous recombination (21). The fact that two mothers with pseudo-PSP presented equally deficient $G_s$ activity compared to their six offsprings with PSP suggests that $G_s$ deficiency may be necessary, but not sufficient, for the development of clinically overt hypoparathyroidism and hypothyroidism. Serum levels of PTH and calcium have been normal in the two mothers between their ages of 36–58 yr and 34–56 yr, respectively. It seems improbable that secondary hyperparathyroidism would develop at a later age.

Circulating bioactive and immunoreactive PTH levels are normal or raised in PSP, but not in pseudo-PSP (2). Plasma from patients with PSP revealed higher PTH inhibitory activity, assessed in a renal cytochemical bioassay, than that in their mothers with pseudo-PSP presented here (22). Intact PTH was separated from a putative inhibitor on gel permeation chromatography of plasma samples. A postulated PTH antagonist whose structure remains to be elucidated may be responsible for the renal resistance to PTH observed in PSP. Yet, some patients with PSP and secondary hyperparathyroidism have osteitis fibrosa (23).

In conclusion, the hypothesis of target organ resistance to PTH being caused by an inactivating mutation of one $G_s\alpha$ allele is questionable in the kindred reported here.

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